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## **Mucosal immune response in common carp** host pathogen interactions in relation to -glucan stimulation

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Mucosal immune response in common carp - host pathogen interactions in relation to  $\beta$ -glucan stimulation.

The overall theme of the PhD study is to investigate and understand the impact of  $\beta$ -glucan as immune modulator in relation to the mucosal immune response (mucus, skin, gills, gut), especially with focus on host – pathogen interactions of freshwater fish with common carp (*Cyprinus carpio* L.) as the model animal. The host pathogen models are based on two common freshwater fish pathogens: The gram negative bacteria *Aeromonas hydrophila* and the ectoparasite ciliate *Ichthyophthirius multifiliis*. Infection with *I. multifiliis* will be introduced in aquarium by co-habitation or by direct infection where theronts will be applied to the water. The parasite life cycle is maintained on rainbow trout as this ensures a slow parasite life cycle, as temperature can be kept low. This will in turn reduce the number of animals used to maintain the parasite life cycle. Infection with *A. hydrophila* will be carried out by intestinal intubation and interpretational injection to ensure a controlled systemic infection. To initiate investigation of skin and mucosal immune reactions, immersion infection will be applied. Diverse ways and doses of  $\beta$  – glucan administration (intraperitoneal injection, bathing, and oral) will be investigated in relation to pathogen infections and resistance.

In the context of infection, expression of immune related genes (IL-1 $\beta$ , IL-6 like, TNF- $\alpha$ , IL-8) will be investigated. The methodology of the project involves the usage of real-time quantitative PCR to quantify expression of genes of interest (the quantitative RT-PCR will be performed by using a Stratagene MX 3000P<sup>TM</sup> real – time system) and an enzyme-linked immunosorbent assay (ELISA) will be used to detect the presence of the natural and specific antibodies in the plasma and mucus from fishes.

Up until now three bath experiments have been carried. Different doses and  $\beta$ -glucans were tested – insoluble Macrogard and soluble, different sized  $\beta$ -glucans. The gill and skin samples were collected and  $\beta$ -glucans effect on IL-1 $\beta$  and IL-6 expression was investigated. Preliminary results from the different sized  $\beta$ -glucans experiment showed that only small (6.3kDa), unbranched  $\beta$  – glucan causes immune response in skin and gill, while 12kD, 15kD, 30kD did not. However, the increased of IL-6 like molecule was observed after 24h and no changes in IL-1 $\beta$  expression were observed. In the next experiment, the effect of Macrogard and 6.3kD  $\beta$ -glucans was investigated on the wounded fish. Wounds were taken by biopsy punches and organs (skin, muscles and gill) were collected at 24h, 3d and 2w after wounding. Preliminary results showed that both  $\beta$ -glucans, Macrogard and soluble 6.3kD sized have an effect on IL-1 $\beta$  and IL-6 expression in skin and gill. However, 6.3kD  $\beta$ -glucan seems to play a role in shift to anti-inflammatory response in the local inflammation.